REMARKS

Claims 1-44 and 51-61 are pending. Claims 2-5, 10-20, 23-27, 29-44, and 51-60 are withdrawn from consideration. Claims 1-5, 10-14, 23-27, 31-35, and 37-61 have been canceled. Claims 6-9, 15-22, 28 and 29 have been amended. Claims 6-9, 15-22, 28-30 and 36 remain in the case.

A request for reconsideration and review is pending, vis-à-vis a decision of December 12, 2002, on applicants' petition for withdrawal or modification of the restriction requirement. Pursuant to the mandate for a complete response, applicants present the following remarks, relating to claim revisions proffered above, but they do not waive their position set out in the request for reconsideration, for which a favorable disposition again is requested.

Responsive to the restriction requirement in question, applicants have canceled 1-5, 10-16, 19, 20, 23-27, 31-35 and 37-61. The basis for restriction between Groups V and X was that the nucleic acid claims read on "DNA encoding a (any) derivative that induces apoptosis and Oltvai *et al.* teach DNA molecule encoding a protein (derivative) that induce apoptosis." The claims have been amended to recite nucleic acid molecules that correspond substantially to SEQ ID NO:10 or that have at least about 45% or greater similarity to SEQ ID NO:10. Oltvai *et al.* disclose a protein that has less then 15% identity to the SEQ ID NO:10. Accordingly, the claims as amended recite a technical feature that defines a contribution over the molecule disclosed in Oltvai *et al.* The basis for restricting between the nucleic acid and protein claims has been obviated, and therefore examination of Groups V and X together is warranted. Reconsideration and withdrawal of the restriction between Groups V and X is respectfully requested.

Claims 6-9, 21, 22, 28 and 61 are objected to because they have not been amended to reflect the elected invention. The claims have been so amended. Claim 61 is objected to as depending on a non-elected claim and has been canceled.

The specification has been amended to remove the embedded hyperlink on page 20, and to remove the reference to Table 1 on page 63.

Claims 6-9, 21, 22, 28 and 61 are rejected under the second paragraph of Section 112, as being indefinite. The claims have been amended to address the concerns raised by the examiner. Reconsideration and withdrawal of the rejection under the second paragraph of Section 112 is respectfully requested.

Claims 1, 6-9, 21, 22 and 28 are rejected under the first paragraph of Section 112, on "written description" grounds. Thus, the examiner contains that the rejected claims contain subject matter that the specification does not describe in such a way as to reasonably convey to one skilled in the art that, at the time the invention was filed, the inventors possessed the claimed invention.

In this regard, the examiner notes that both human and murine Bim genes encode three different products, and he alleges that these products differ in both molecular structure and biological function. To substantiate the contention of differing biological function, the examiner invokes data in applicants' specification to urge that the degree of apoptosis varies among the various species. This in no way contravenes applicants' teaching and corresponding claim recitation, however, which is characterized by the ability to induce apoptosis.

The examiner also asserts that data in the specification, said to show that apoptosis is induced, relate to Bims and Bim$_L$ rather than to Bim$_{EL}$, the elected species. Attached to this response, however, are data demonstrating the binding properties in Table A and killing activity of Bim$_{EL}$. The data clearly show that Bim$_{EL}$, as taught in the specification, induces apoptosis, which is referenced as "killing activity" in Table B. The data further show that human Bim$_{EL}$ loses its apoptosis-inducing activity when the BH3 domain is deleted. In other words, a significant level of apoptosis-inducing activity is localized to the BH3 region. Furthermore, a variant of Bim$_{EL}$ has been generated that has lost the ability to bind to the dynein light chain. This variant exhibits improved apoptotic activity.*

* If requested, applicants will provide a declaration under Rule 132 which includes the referenced data.

The assays used to test the functional activity of these molecules are described briefly in the figure legend. More detailed descriptions of the assays appear in Puthalakath *et al.*, *Molecular Cell* 3: 287 (1999), and O'Connor *et al.*, *EMBO J.* 17: 384 (1998), copies of which accompany this response. These are standard assays for apoptosis induction and were well known before the priority date of the present application. The appended data provide conclusive evidence that (i) the human Bim$_{EL}$, according to SEQ ID NO:10, induces apoptosis, and (ii) variants of Bim$_{EL}$, generated in accordance with the present specification, function even better with respect to inducing apoptosis than the parent molecule.

Claims 21, 22 and 28 also stand rejected for alleged lack of enablement, with the examiner contending that data cannot be extrapolated, given the fact that even slight variations in protein's structure can have a significant and unpredictable effect on biological activity. The manufacture of other variants consistent with applicants' teachings, however, and their testing using the well known assays referenced above is well within the ambit of routine skill in this art and, hence, does not require experimentation that is "undue," within the meaning of the "enablement" provision of Section 112. More particularly, a skilled technician would be able to make nucleic acids that encode polypeptides based on SEQ ID NO:10 in which at least one amino acid is added, substituted or deleted, and then test the results using the available assays. This is borne out by the appended data.

The examiner comments on the correlation between binding to the dynein light chain and functionality. In particular, she notes that "the specification says that BH3 domain is essential for apoptosis," citing Example 7. The relevance of this relationship between the BH3 domain and the dynein light chain binding region of Bim are clearly disclosed in the specification, and it is submitted that the examiner may have misapprehended the import of Example 7. Although this example shows that BH3 plays a significant role in promoting apoptosis, at page 64, lines 29-30, it clearly is stated that Bim$_{L}$ in which the BH3 domain is deleted is *not completely inactive*. The top of page 65 goes on to state that "thus, regions of Bim other than BH3 may promote apoptosis or interfere with clonogenicity in another

way." It is for this reason that the mutant which exhibits the deletion of the BH3 domain still retains some apoptotic activity.

The comments in the Action suggest that the examiner may not fully appreciate the functional significance of the BH domain and the dynein light chain binding domain in the context of various Bim isoforms. The BH domain, and more particularly the BH3 domain, are important with respect to Bcl-2 binding and therefore impact apoptosis by blocking the functioning of the pro-survival molecule Bcl-2. The dynein light chain domain binds to Bim. This prevents Bim from binding to Bcl-2 and preventing the pro-survival functioning of Bcl-2. In other words, the specification teaches that modulation of the dynein light chain region so that the Bim molecule can not longer bind can be used to design of variants optimized for apoptosis functionality.

The examiner further asserts that the specification does not teach any other structures responsible for apoptosis nor provide guidance as to what changes can be made to retain apoptotic activity while abolishing the dynein light chain ability of human Bim$_{EL}$. However, the specification does provide an explicit teaching in this regard at page 36 lines 20-22, which states that the region of the human Bim$_{EL}$ amino acid sequence which binds the dynein light chain is defined by residue numbers 42-131. Accordingly, the specification clearly teaches which mutations would be required in order to abolish dynein light chain binding. The specification further teaches at page 36, lines 4-10, that a Bim molecule which is unable to bind with dynein light chain is thereby free to interact with Bcl-2 and prevent its pro-survival function. Accordingly, this clearly teaches that a variant of Bim$_{EL}$, mutated in the amino acid residue region 42-131 such that it does not bind the dynein light chain, will inherently exhibit improved apoptotic inducing activity due to its ability to interact with and thereby inhibit the pro-survival molecule Bcl-2. This is confirmed by the appended data.

Finally, the examiner comments on disclosure in the specification that the biological functions of one splice variant of Bim are not the same as those of other splice variants, with longer splice variants having all of the amino acid sequence of shorter splice variants, but with the shorter splice variants having the best apoptotic activity. The reason that the

shorter splice variant has the best apoptotic activity is that this variant does not exhibit a dynein light chain binding region. This is disclosed in the specification, and further supports the notion that in designing variants to fall with the scope of the claims one would direct the variations to the disclosed dynein light chain binding region and seek to abolish the activity of this region to bind with this molecule, thereby freeing the Bim variant to bind to and inhibit Bcl-2.

Claims 1, 6-9, and 61 are rejected under the first paragraph of Section 112. The examiner states that the specification enables the production and use of SEQ ID NO:9 DNA that encodes SEQ ID NO:10, a human Bim$_{EL}$ that is able to induce apoptosis, but does not enable "any other isolated DNA molecules." As noted above, the specification clearly provides guidance in this regard, enabling a skilled technician to make other DNAs and to implement assays to test the pro-apoptotic functional activity of these molecules. Moreover, the specification discloses results with other structurally similar molecules, human Bim$_L$, which differs from human Bim$_{EL}$ slightly in length, and murine Bim$_S$, Bim$_L$, and Bim$_{EL}$, which also are highly similar to Bim$_{EL}$. Murine and human Bim$_{EL}$ molecules exhibit 87% identity at the amino level, which is significantly higher than the closest prior art molecules identified via a Blast search, which exhibited less than 15% amino acid identity. Accordingly, the specification discloses a family of five Bim molecules that exhibit significant structural similarity at the protein level and, inherently, significant similarity at (i) the level of nucleic acid molecules which encode these proteins and (ii) the functional level.

The examiner alleges that the specification teaches Bim genes encoding different products with different molecular structures and different biological functions. To the contrary, the specification discloses a family of molecules that exhibit the same primary biological function, the ability to induce apoptosis. Further, the only significant structural difference between these molecules is the absence, in the case of Bim$_S$ molecule, of a dynein light chain binding region. The absence of this region facilitates interaction of Bim$_S$ with Bcl-2, thereby promoting a high level of apoptotic activity due to inhibition of Bcl-2 functioning. The structurally and functionally related family of Bim molecules taught by

applicants provides significant information in relation to the structure of these molecules in terms of functional impact. The specification also provides sufficient teaching to enable one to routinely design and perform procedures which would enable the expression of a nucleic acid sequence and testing of the pro-apoptotic functional activity of the protein products produced thereby.

On page 12 of the Action, the examiner states that the specification does not teach any method of using any peptide encoded by SEQ ID NO:9 that does not possess apoptotic activity. This statement is not understood, as applicants' claims do not encompass peptides that do not exhibit apoptotic activity.

Claims 1, 6-9, 21, 22, 28 and 61 are rejected under Section 101. The claims have been amended to recite "isolated" nucleic acids and polypeptides, obviating this rejection.

Claims 1 and 61 are rejected under Section 102(b) based on Oltvai *et al.* Oltvai *et al* does not disclose sequences as recited in the amended claims. Reconsideration and withdrawal of this rejection is requested.

In view of the foregoing amendments and remarks, it is believed that all claims are in condition for allowance. Reconsideration of all rejections and a notice of allowance are respectfully requested. Should there be any questions regarding this application, the examiner is invited to contact the undersigned attorney at the phone number listed below.

Respectfully submitted,

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MARKED-UP VERSION SHOWING CHANGES MADE – SPECIFICATION

Please amend the last paragraph on page 20 as follows:

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particular preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of Needleman and Wunsch. Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on the ANGIS (Australian National Genomic Information Service) [at] website [http://mell.angis.org.au].

Please amend the first paragraph on page 63 as follows:

We next explored whether all isoforms of Bim were equivalent. An FDC-P1 clone expressing human Bcl-2 was transfected with vectors expressing Bim_{EL}, Bim_{I} or Bim_{S} and puromycin-resistant clones that expressed the same amount of each isoform were selected for further analysis (Figure 6A). To test for association with Bcl-2, immunoprecipitates prepared from cell lysates using a monoclonal antibody specific for human Bcl-2 were fractionated electrophoretically and blotted with anti-EE antibody. Each of the Bim isoforms clearly bound to Bcl-2 (Figure 6B). However, when the transfectants were deprived of IL-3 or subjected to γ-irradiation, it became evident that Bim_{S} antagonised Bcl-2 more effectively than Bim_{L} while Bim_{EL} was the least potent (Figures 6C). In addition, Bim_{S} suppressed L929 colony formation more effectively than Bim_{L} or Bim_{EL} [(Table 1)].

Thus, although all three Bim isoforms can bind to Bcl-2, they vary in cytotoxicity, Bims being the most potent.

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MARKED-UP VERSION SHOWING CHANGES MADE – CLAIMS

Please cancel claims 1-5, 10-14, 23-27, 31-35, and 37-61 and amend the remaining claims as follows:

6. (Amended) [A] An isolated nucleic acid molecule [according to claim 1 wherein said nucleic acid molecule comprises] comprising a nucleotide sequence encoding or complementary to a sequence encoding an amino acid sequence [substantially as set forth in one] of SEQ ID NO:[8 or] 10 [or a derivative or homologue thereof] or having at least about 45% or greater [similarity] identity to [one or more of] SEQ ID NO:[8 or] 10 [or derivative or homologue thereof] wherein said amino acid sequence is characterized by the ability to induce apoptosis.

7. (Amended) [A] An isolated nucleic acid molecule [according to claim 1] comprising a nucleotide sequence [substantially as set forth in] of SEQ ID NO:[7 or] 9 [or a derivative or homologue thereof] or capable of hybridising to [one of] SEQ ID NO:[7 or] 9 under [low] moderate stringency conditions [at 42°C] wherein said nucleic acid molecule encodes a polypeptide characterized by the ability to induce apoptosis.

8. (Amended) [A] An isolated nucleic acid molecule according to claim 7 which further encodes an amino acid sequence corresponding to an amino acid sequence [substantially as set forth in one] of SEQ ID NO:[8 or] 10 [or a derivative or homologue thereof] or having at least about 45% or greater [similarity] identity to [one or more of] SEQ ID NO:[8 or] 10 [or a derivative of homologue thereof].

9. (Amended) [A] An isolated nucleic acid molecule according to claim 7 [substantially as set forth in one] of SEQ ID NO:[7 or] 9.

15. (Amended) [A] An isolated polypeptide [according to claim 10] comprising an amino acid sequence [substantially as set forth in] of SEQ ID NO:[8 or] 10 [or derivative or homologue thereof] or a sequence having at least about 45% [similarity] identity to [one or more of] SEQ ID NO:[8 or] 10, wherein said polypeptide is characterized by the ability to induce apoptosis.

16. (Amended) [A] An isolated polypeptide according to claim [10] 15 encoded by a nucleotide sequence [substantially as set forth in] of SEQ ID NO:[7 or] 9 [or a derivative or homologue thereof] under [low] moderate stringency conditions [at 42°C].

17. (Amended) [A] An isolated polypeptide according to claim 16 further comprising an amino acid sequence [substantially as set forth in] of SEQ ID NO:[8 or] 10 [or derivative or homologue thereof] or a sequence having at least about 45% [similarity] identity to [one or more of] SEQ ID NO:[8 or] 10.

18. (Twice Amended) [A] An isolated polypeptide according to claim 16 [substantially as set forth in] having SEQ ID NO:[8 or] 10.

19. (Twice Amended) [A] An isolated polypeptide according to claim [10] 15 in homodimeric form.

20. (Twice Amended) [A] An isolated polypeptide according to claim [10] 15 in heterodimeric form.

21. (Twice Amended) A variant of an isolated [*Bim*] nucleic acid molecule as claimed in claim [1] 6 comprising one or more nucleotide mutations in said nucleic acid molecule resulting in at least one amino acid addition, substitution and/or deletion to the polypeptide encoded by said variant wherein said polypeptide cannot bind, couple or otherwise associate with a dynein light chain and wherein said polypeptide is characterized by the ability to induce apoptosis.

22. (Amended) A variant according to claim 21 wherein said mutation results in an amino acid addition, substitution and/or deletion in the region of the polypeptide chain which binds the dynein light chain.

28. (Amended) A variant according to claim 22 wherein said [*Bim*] nucleic acid molecule is human *Bim*EL and said region is defined by amino acid residue numbers 42 to 131.

29. (Amended) A variant of an isolated [Bim] polypeptide as claimed in claim [10] 15 comprising at least one amino acid addition, substitution, and/or deletion wherein said variant cannot bind, couple or otherwise associate with the dynein light chain.